10 7 8
--29. A method according to claim 26 or claim 27, wherein said antibody is conjugated to a cytotoxic material.--

11 10
--30. A method according to claim 29, wherein said cytotoxic material is selected from the group consisting of ricin A chain, diphtheria toxin, Pseudomonas exotoxin A and idarubicin.--

C1

12 7 8
--31. A method according to claim 26 or claim 27, wherein said antibody is conjugated to a radioisotope label.--

13 12
--32. A method according to claim 31, wherein said radioisotope label is technetium-99m.--

REMARKS

Responsive to the Office Action of January 23, 1998, the title of the application has been amended, Claims 17-19 have been canceled, and new claims 20-32 have been added to more particularly point out and distinctly claim the subject matter of the claimed invention. Claims 20, 26 and 27 correspond generally to the subject matter of former claims 17, 18 and 19, respectively. Favorable reconsideration of the application is therefore respectfully requested.

The title of the application was objected to as being not descriptive, and the title has now been amended to reflect that the claimed invention is drawn to treatment methods and to

19

antibody compositions for such therapies, in accordance with the Examiner's suggestion.

Claims 17-19 were rejected on the grounds that the recitation of "label" was indefinite. Claims 20, 26 and 27 now recite a "detectable label" in accordance with the Examiner's suggestion.

Claims 17-19 were also rejected on the grounds that they were non-enabling for *in vivo* methods of inhibiting angiogenesis and treating tumor associated angiogenesis in human patients comprising the administration of antibodies that bind to proliferating human endothelial cells. The claims were asserted to pertain to "the highly experimental and unpredictable field of *in vivo* human therapy using monoclonal antibodies," and the Harris, Hird and Dillman references were cited to highlight the problems associated with the use of monoclonal antibodies for the treatment of human cancers *in vivo*.

It is respectfully submitted that the monoclonal antibodies of the present invention are very different from those that have been used in the past to treat human beings *in vivo*. The major difference in this regard is that the present antibodies target the endothelial cells of the blood vessels within tumors, and not the tumor cells themselves. It is respectfully submitted that the tumor endothelial cells are a very attractive target as they are freely accessible to the antibodies, are genetically stable, and express unique antigens when they are in an angiogenic state. A review by Thorpe and Burrows in 1995 (*Breast Cancer Res. Treat.* **36**:237) highlights this point. A copy of this review will be forwarded for the Examiner's consideration as soon as is possible. Based upon this review and the work of others, it is now well accepted by workers in the field that monoclonal antibodies specific for proliferating endothelial cells have considerable

therapeutic potential in cancer patients. In particular, these antibodies can be used to target cytotoxic drugs to the vasculature and inhibit angiogenesis.

Matsuzaki (PNAS 86:9911, 1989) was cited by the Examiner for the proposition that monoclonal antibodies that inhibit growth *in vitro* of endothelial cells did not inhibit *in vivo* growth of tumors, and did not prevent the growth of highly vascularized tumors *in vivo*. However, the compositions and methods of the invention do not target a particular growth factor as was investigated in Matsuzaki, but rather target specific types of proliferating endothelial cells. It is thus respectfully submitted that findings of Matsuzaki are inapposite to the present invention.

The Examiner also asserts that "there is little future for the use of rodent monoclonal antibodies for *in vivo* human therapy." It is acknowledged that there may be complications associated with the use of rodent monoclonal antibodies, but the production of humanized versions of these rodent antibodies is now standard practice in the field.

The Examiner notes with regard to former claims 18 and 19 concerning the treatment methods that the specification teaches only three antibodies with specificity for proliferating human endothelial cells which inhibit the proliferation of HUVE cells cultured *in vitro*. The Examiner asserted that the specification does not provide sufficient evidence to give one skilled in the art a reasonable expectation of using the antibody compositions and methods of claims 18 and 19 to effectively inhibit angiogenesis-dependent tumor growth. It is respectfully submitted that new claims 20, 26 and 27 now specifically recite the antibody which specifically binds proliferating human endothelial cells in the same terms as in parent application, now patent

number 5,67,181, namely as binding proliferating HUVE or HUAE cells and not binding non-proliferating HUVE and HUAE cells. Accordingly it is respectfully submitted that the claims as amended are no longer directed to antibodies with a general specificity for proliferating human endothelial cells, and bearing in mind this limitation in the scope of the claims, it is respectfully submitted that the specification for the present invention does provide sufficient evidence to give a person in this art a reasonable expectation of using the claimed compositions and methods to inhibit or treat tumor associate angiogenesis.

The Examiner further suggested that the specification provides "no guidance for selecting antibodies that can effectively be used in methods of treating tumor associated angiogenesis and inhibiting angiogenesis." However, the flow cytometry-based screening method used by the present invention screens for antibodies that fail to react with resting endothelial cells obtained from the human umbilical vein but detects antibodies that react with the cell surface of proliferating endothelial cells from the same vascular source. Such antibodies would be expected to be excellent candidates for targeting antibody-immunotoxin conjugates to angiogenic sites in tumors *in vivo*. Effective antibody-immunotoxin conjugates which can eliminate proliferating endothelial cells can be readily screened for *in vitro* activity prior to use *in vivo*. It is respectfully submitted that this is common practice in the field, and overcomes the variability problems described by Press et al. (*J. Immunol.* **141**:4410, 1988) as cited by the Examiner.

The Examiner asserted that the specification does not set forth sufficient direction or guidance to enable one skilled in the art to effectively practice the claimed methods, on the

grounds that the specification provides no detailed description of how to effectively administer monoclonal antibodies, particularly with regard to effective dosages which are critical to achieving "an effective therapeutic result."

In the specification at page 6, lines 11-18, it is indicated that 50 μl/well of hybridoma supernatant was added to each assay of $2.5x10^4$ cells. As summarized at page 9, lines 22-24, if was found that HUVE cell proliferation was inhibited by some of the assayed monoclonal antibodies at this dosage. It is therefore clear that sufficient direction and guidance are sufficiently described, and that adequate examples are given to enable one of ordinary skill in the art to determine without undue experimentation which monoclonal antibodies will work, and effective dosages in utilizing the claimed invention.

It is further noted that Claim 20 recites "A therapeutic composition for inhibition of tumor associated angiogenesis or for treatment of tumor associated angiogenesis," and does not recite a therapeutic amount.

The Office Action further stated that no working examples were provided that would provide sufficient guidance to allow one skilled in the art for use of antibodies with the claimed specificity for the effective treatment of angiogenesis-dependent diseases with a reasonable expectation of success. A detailed example of effective administration of monoclonal antibodies is provided at pages 5 - 10 of the specification, sufficient to enable one skilled in the art to practice the invention as claimed.

The Examiner finally concluded that "undue experimentation" would be required to practice the claimed methods with a reasonable expectation of success. It is respectfully

submitted that in view of the detailed example given in the specification as to dosage of monoclonal antibodies in successful application of the method of the invention, any experimentation that may be required for a application of the methods of the invention in the particular circumstances of a given situation would be routine experimentation, and not undue experimentation. It is respectfully submitted that successful administration of monoclonal antibodies in prophylactic and therapeutic treatments is now well known, and the determination of, for example, effective dosages which are necessary in order to achieve a particular result can be carried out by simple and routine trial, which would not amount to undue experimentation.

Concerning the prior art rejections of Claims 17-19 set out in paragraphs 5-11 of the Office Action, it is respectfully submitted that claims 20, 26 and 27 now recite a specific definition of the antibodies restricting the claims to antibodies binding proliferating HUVE or HUAE cells and not binding non-proliferating HUVE and HUAE cells. Thus, the antibodies of Springer ('660) specifically bind to human ICAM-2 are clearly not specific for proliferating human endothelial cells. The ICAM-2 molecule is constitutively expressed by all endothelial cells, and therefore does not meet the criterion of only being expressed on proliferating endothelial cells. Similarly, in Brodsky et al. (1979), the monoclonal antibodies disclosed are specific for major histocompatibility complex antigens, and are not appropriate for targeting proliferating human endothelial cells. The MHC antigens are expressed on all endothelial cells and not exclusively on proliferating human endothelial cells. On the same basis, the rejection of claim 17, on the grounds of obviousness in view of Gougos, is not applicable in relation to the amended claims now proposed. Gougos merely discloses the use of 44G4 antibody which binds

to endothelial cells in general. Similarly, the rejection of claim 17 on the grounds of obviousness in view of Wang et al., in view of Harlow and Lane, is not applicable in relation to the amended claims since the antibodies disclosed by Wang et al. do not react exclusively with proliferating human endothelial cells.

Claims 17-19 were further rejected as being anticipated by Hagemeier et al. Although the monoclonal antibody EN 7/44 is described in Hagemeier et al. as reacting with cultured HUVEC, it was also shown to react with umbilical vein endothelial cells in tissue sections of the umbilical cord by immunohistochemistry. Thus, the monoclonal antibody EN 7/44 is not specific for proliferating HUVEC. In addition, the Hagemeier et al. monoclonal antibody fails to react with cell surface molecules on HUVEC and only reacts with a cytoplasmic antigen. Thus, one would anticipate that the monoclonal antibody would not be an effective antibody for targeting proliferating endothelial cells in blood vessels. The antibodies of the present invention react with cell surface antigens on endothelial cells, and are thus able to target cytotoxic drugs to the vasculature in proliferating cell sites.

Claims 17 to 19 were also rejected under the judicially created doctrine of obviousness type double patenting in view of U.S. Patent No. 5,677,181. It is respectfully submitted that in view of the enclosed Terminal Disclaimer, the rejection under the judicially created doctrine of obviousness type double patenting should now be withdrawn.

Our check in the amount of $55.00 is enclosed to cover the fee for filing the disclaimer.

In light of the foregoing, it is respectfully submitted that the application should now be in a condition for allowance, and an early favorable action in this regard is respectfully requested.

Respectfully submitted,

FULWIDER PATTON LEE & UTECHT, LLP

David G. Parkhurst
Registration No. 29,422

DGP/mem
Enclosures:
Terminal Disclaimer
Transmittal Form PTO/SB/21
Fee Transmittal PTO/SB/17
Check for $55.00
Return Postcard

10877 Wilshire Blvd., Tenth Floor
Los Angeles, CA 90024
Tele. No. (310) 824-5555
Facsimile No. (310) 824-9696